UNITED STATES DEPARTMENT OF AGRICULTURE

ENZYME INACTIVATION TESTS (FROZEN VEGETABLES)

| Agricultural |
|--------------|
| Marketing |
| Service |

Fruit and Vegetable Division

Processed Products Branch TECHNICAL INSPECTION PROCEDURES

Washington, DC

File Code 135-A-12

November 1975

I PURPOSE

This instruction outlines Branch policy on enzyme inactivation tests on frozen vegetables. It prescribes where tests should be made, rates of analyses and methods of testing.

II POLICY

The Branch will consider the peroxidase test to be the official test for enzyme inactivation.

III INTRODUCTION

Research studies have demonstrated that there is close inverse relationship between the degree of enzyme activity and quality retention during storage. Off-color and off-odors can be associated with certain enzyme systems not sufficiently inactivated by blanching at time of pack.

In the case of frozen vegetables the two common enzymes that require inactivation are the <u>catalase</u> system and the <u>peroxidase</u> system.

While there is some disagreement among scientists and food processors concerning the most appropriate method of determining adequacy of blanch, it is generally agreed that total destruction of the enzyme is not necessary. Furthermore, present knowledge indicates that the peroxidase system is somewhat more heat tolerant and if it is inactivated the chances are that the catalase system is also inactivated.

Irrespective of the merits of blanching to inactivate one system in preference to the other this instruction presents procedures that have been found practical in testing the end product for inactivation of enzymes.

Proper blanching of a vegetable prior to freezing will inactivate the enzymes which would otherwise cause development of off-flavors, loss of color and loss of Vitamin A and C during storage after freezing. Blanching also tends to fix the characteristic color of a vegetable. Overblanching may cause dull color, flavor loss, sloughing, and poor texture. In achieving the same degree of enzyme inactivation, better color retention is attained by blanching at a higher temperature for a short time, than through lower temperatures for a longer time.

For the majority of vegetables, inactivation of catalase alone is not an indication of adequate blanch. Inactivation of the peroxidase enzyme is considered necessary to minimize the possibility of future deterioration in quality. Thirty to fifty percent more blanch time is required to inactivate peroxidase than would be required to inactivate catalase alone. The catalase test is also a poor indication of blanch on products after they have been in storage for several months, since there is a gradual disappearance of catalase activity.

IV INSPECTION PROCEDURES

A. <u>Lot Inspection</u>

Products inspected under lot inspection will not routinely be tested for enzyme inactivation. The peroxidase test will be made on all condition inspections, or if required by Federal or buyer's specifications, or if requested by the applicant. In addition, the peroxidase test will be run during routine inspections of frozen vegetables if there is organoleptic evidence of under-blanch.

Tests for peroxidase are frequently required on supplies going to the military and on other buyer's specifications. When specifications call for catalase or tests other than the peroxidase test or specify methods other than the USDA methods contained in this File Code, such tests should be performed as required by the specification.

When it is <u>required</u> that enzyme inactivation tests be performed, the minimum rate is:

| Quality | Number |
|-------------|----------|
| Sample Size | of Tests |
| | |
| 3 | 1 |
| 6 | 1 |
| 13 | 3 |
| 21 | 3 |
| 29 | 4 |
| 38 | 5 |
| 48 | 6 |
| 60 | 7 |

Additional tests should be made if organoleptic inspection indicates any possible inadequate blanching.

B. <u>In-Plant Inspection -- OfficialENDFIELD</u>

All vegetables except onion rings, sweet peppers, rhubarb, french fried potatoes and cooked squash processed and frozen under in-plant inspection are to be tested for peroxidase inactivation. Testing for catalase inactivation, where required by Federal, buyer's or other specifications, must be in addition to the peroxidase test. Testing using methods outlined in specifications is permissible in lieu of the USDA method when inspection and certification are based on such specifications. Samples from each blancher are to be tested at the beginning of every processing period. Additional sample units should be tested whenever there is an indication that the blanch may have changed. Tests should also be made at least hourly throughout the shift.

C. Quality Control Testing - Unofficial

The official peroxidase test as well as other enzyme inactivation tests are useful in quality control. For example, blanch temperature may be adjusted so that a peroxidase test will show no color development within three and a half minutes but will show color development within eight minutes. It is also useful to test the larger units of product for peroxidase activity. If there is color development in two and a half or three minutes, depending upon the variation in unit size of the product, the product may be considered neither under nor over blanched and official tests with units representative of all unit sizes may remain negative. Low initial blanch temperature, drops in temperature, increased volume of product or increase in unit size of product should be regarded as indications of possible under blanching and usually suggests additional quality control checks. Such quality control tests from isolated portions of production can and should be made for those plants relying on the inspector for help in quality control. This should be noted on the score sheet, but should not be considered as the official routine peroxidase test unless representative of the production. Similarly, quality control tests on larger units may be recorded on the score sheets, but this should be noted as being a restrictive test and not one of the routine representative tests for the hour.

Quality control tests may be made on the cut surface of an individual unit of product, but these are not considered as official tests. Pieces of the product can be tested with 0-tolidine paper and hydrogen peroxide to show the extent of any unblanched area in a piece and to show variations among pieces of the same size to judge the uniformity of the blanch.

SPECIAL TESTS FOR CERTAIN PRODUCTS

- Broccoli For quality control purposes testing the top center portion of the heads of the large stocks where enzyme activity, if present, will be concentrated. This may give quality control additional information, but is not to be considered an official test.
- 2. <u>Brussels Sprouts</u> The rapid development of pink color after about 15 seconds with the addition of 1/2 to 3 percent H(2)0(2) to the cut surface of units immediately after blanching, or development of pink centers soon after freezing and storage are valuable quality control checks. Correlation of this test with the official test should provide a rapid quality control aid.

3. <u>Corn-on-the-Cob</u> - When testing corn-on-the-cob use only the kernels removed from the cob. Scrapings taken from the cob at the base of the kernels may show residual peroxidase activity although the product is adequately blanched. Victor Golden and certain other varieties contain larger amounts of enzyme than Golden Cross and other varieties and usually requires longer than the normal blanch.

V SIZE, COLLECTION AND HANDLING OF SAMPLE UNITS

Use a 200 gram sample that is representative of the variation in piece sizes. If some pieces are very large, all pieces shall be cut in quarters and a quarter from each piece used for the 200 gram sample.

Blanched, cooled, unfrozen samples should be taken just before entering the freezer. Test within 1/2 hour, or quickly cool to 35 degrees F. in ice water or in a freezer compartment and run within 2-3 hours.

Frozen samples should be water thawed in tap water at no more than 86 degrees F. until units can be easily separated. Do not perform test on completely frozen sample unit. ENZYME TESTS MUST BE COMPLETED WITHIN 30 MINUTES OF THAWING.

VI TEST PROCEDURE

A. Peroxidase Test

The test for adequacy of blanching in frozen vegetables based upon inactivation of the peroxidase system outlined herein:

1. Reagents

Distilled Water.

0.5 percent guaiacol in 50 percent ethyl alcohol solution or a 0.5 percent guaiacol in 50 percent 2-propanol (isopropyl alcohol) solution.

0.08 percent hydrogen peroxide (2.8 ml. of 30 percent hydrogen peroxide made up per liter, using distilled water). Keep in refrigerator in dark bottle and renew each week or two.

2. Apparatus

Test tubes - 3/4 or 7/8 inch diameter

Funnels - 3 or 4 inch diameter

Cotton milk filters - 6 or 7 inch diameter or SS 604 filter paper or similar quality, 18.5 cm. in diameter.

Mechanical blender - Waring or similar type

Graduated cylinder - 50 ml.

Pipettes - 1 ml. and 2 ml.

Timer or watch with second hand

Test tube rack

Balance with accuracy of "plus or minus" 0.1 gram

3. <u>Procedure</u>

- a. Weigh out representative 200 gram sample. (See Section V, Size, Collection and Handling of Sample Units.)
- b. Complete the test within 30 minutes after the product is thawed.
- c. Place in blender with 600 ml. of water.
- d. Macerate for 1 minute at high speed.
- e. Filter through cotton milk filter. If cotton milk filter is not available, SS 604 filter paper may be used. Discard the first 5-10 ml. of filtrate.
- f. Prepare a blank by adding 21 ml. of distilled water to 2 ml. of filtrate in a test tube. Then add 1 ml. of 1/2 percent guaiacol solution without mixing. (Do not add any hydrogen peroxide to this tube.)
- g. Add 20 ml. of distilled water to 2 ml. of filtrate in a second test tube. (See Plate I attached.)

- h. Add 1 ml. of 1/2 percent guaiacol solution to this second test tube (step g) without mixing.
- i. Add 1 ml. of 0.08 percent hydrogen peroxide to the same tube without mixing.
- j. Mix contents of both tubes thoroughly by inverting each 3 times and returning to test tube rack. Watch for development of any color in the tube to which hydrogen peroxide was added. Use the blank tube containing guaiacol, water and filtrate for comparison. Any color change in the sample tube that is in obvious contrast to the blank is considered a positive test. If no such color contrast develops in 3-/12 minutes, 1/2 consider the test negative and the product adequately blanched. If color develops after 3 1/2 minutes, it is to be disregarded, and the test still considered negative.

1/ Zucchini Squash: 1 minute.

Brussels Sprouts: 2 1/2 minutes; however, consider test results on the lot as meeting if no more than 1 of 3 sample units tested change after 2 minutes. A lot fails if any sample units change color before 2 minutes.

B. <u>Catalase Test</u>

The test for adequacy of blanching in frozen vegetables based upon inactivation of the catalase system is outlined herein:

1. Reagents

Distilled water.

Calcium carbonate (Ca CO(3)).

Hydrogen peroxide - 3 percent (10 ml. of 30 percent hydrogen peroxide per 100 ml. of distilled water). Keep in refrigerator in a dark bottle.

2. Apparatus

Mechanical blender - Waring or similar type.

Fermentation tube - on foot with vertical tube graduated from 0 to 5 ml. in 1/10 ml. graduations (Kimble 46162 or similar)

Funnels - 3 or 4 inch diameter.

Cotton Milk filters - 6 or 7 inch diameter or SS 604 filter paper or similar quality. 18.5 cm. in diameter.

Pipettes - 1 ml., 5 ml., and 10 ml.

Balance with accuracy of "plus or minus" 0.1 gram.

Timer or watch with second hand.

3. Procedure

- a. Comminute a 100 gram portion of vegetable material together with 100 ml. of water and about 2 grams of calcium carbonate.
- b. Filter slurry through cotton milk filter.
- c. Add 2 ml. of distilled water to a fermentation tube (See Place II attached).

- d. Pipet 2 ml. of filtrate into tube.
- e. Add 8 ml. of 3 percent hydrogen peroxide to tube.
- f. Invert tube in such a manner as to completely fill the calibrated column. Tap gently to dissipate any bubbles that result from mixing solution.
- g. Return to upright position and allow contents to generate for 3 1/2 minutes.
- h. At the end of 3 1/2 minutes record the volume of gas formed.
- i. Disregard any reading of 0.1 ml. or less. <u>Consider any reading of more than 0.1 ml. as positive.</u>

VI CERTIFICATION

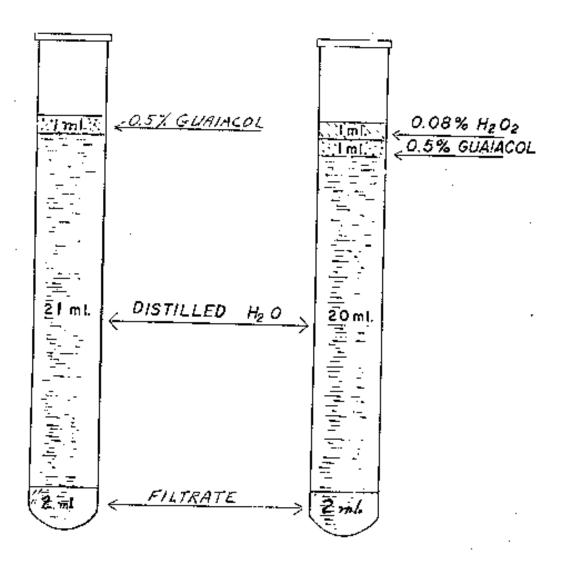
Official peroxidase or catalase tests that are representative of a production period or lot are shown on the score sheets for the item but <u>are not</u> reported on a certificate unless:

- A. Requested by the Applicant.
- B. Required by Federal, Buyer's or other Specification.
- C. Inspection is for condition of the product and some of the samples are positive.

Refer to 165-A-1 for appropriate statements to be shown in the "body" and/or "Grade" section of a certificate.

FILE CODE 135-A-12 NOVEMBER 1975 PLATE I

PEROXIDASE TEST



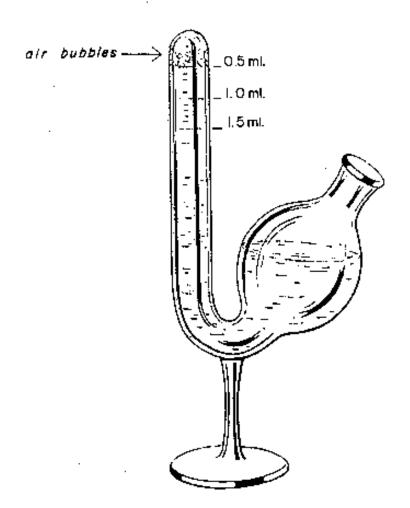
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No. 2
(SAMPLE)

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PLATE II

CATALASE TEST



FERMENTATION TUBE